Journal of Food Engineering 109 (2012) 249–257

Contents lists available at SciVerse ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Supercritical fluid extraction of grape seed: Process scale-up, extract chemical composition and economic evaluation

Juliana M. Prado [a], Irede Dalmolin [a], Natália D.D. Carareto [a], Rodrigo C. Basso [a], Antonio J.A. Meirelles [a], J. Vladimir Oliveira [b], Eduardo A.C. Batista [a], M. Angela A. Meireles [a,*]

[a] DEA/FEA (School of Food Engineering)/UNICAMP (University of Campinas), R. Monteiro Lobato, 80, Campinas, 13083-862 SP, Brazil
[b] Department of Food Engineering, URI–Campus de Erechim, Av. Sete de Setembro, 1621, Erechim, 99700-000 RS, Brazil

ARTICLE INFO

Article history:
Received 22 February 2011
Received in revised form 18 May 2011
Accepted 4 October 2011
Available online 18 October 2011

Keywords:
Economic evaluation
Grape seed
Scale-up
Supercritical fluid extraction

ABSTRACT

The scale-up of the supercritical fluid extraction (SFE) process for grape (*Vitis vinifera* L.) seed was studied from the laboratory (0.29 L) to the pilot scale (5.15 L) at 35 MPa and 313 K. The scale-up criterion adopted consisted of maintaining a constant solvent to feed ratio (S/F), and the criterion was successfully used to predict the approximate behavior of the SFE process from the laboratory at the pilot scale for a 17-fold scale-up. Linoleic acid was the major component of the extract; palmitic, stearic and oleic acids were also detected. The economic evaluation showed that it is viable to establish a SFE plant in Brazil for SFE processing of grape seed. From the technical-economic evaluation, for SFE of grape seed at 313 K/35 MPa, an extraction time of 240 min and S/F of 6.6 produced the best relationship between yield and cost.

1. Introduction

Scale-up criteria described in the literature for the supercritical fluid extraction (SFE) process include maintaining constant kinetic parameters (Martínez et al., 2007; Mezzomo et al., 2009), the development of empirical equations based on bed geometry (Carvalho Jr. et al., 2005; Moura et al., 2005) and the use of mathematical models (Del Valle et al., 2004; Kotnik et al., 2007; Han et al., 2009) to predict the behavior of the process when the scale is increased. However, many of these studies are inconclusive, and there is no consensus in the literature on a scale-up criterion applicable to solid matrices.

Our research group has recently been studying a very simple scale-up criterion, maintaining a constant solvent to feed ratio (S/F), with encouraging results (Prado et al.,2011; Prado and Meireles, 2010). When validating scale-up criteria, it is necessary to assess their applicability to different types of raw materials, as the mass transfer mechanisms may differ among species and parts of the plant used for extraction (Reverchon and Marrone, 1997).

Making use of waste products is a general research trend in the scientific community. In many countries, such as Brazil, grape seeds are considered a disposable waste material by the majority of wineries. They are usually discarded, burned or used as animal feed (Molero Gómez et al., 1996). In the southern region of Brazil, the grape industry plays an important social-economic role (Freitas, 2007); therefore, reusing the residues of this industry is of great interest.

Grape seed oil offers several benefits for human health, because of its high content of unsaturated fatty acids and antioxidant compounds such as monomeric flavan-3-ols, phenolic acids and oligomeric proanthocyanidins (Molero Gómez et al., 1996; Jayaprakasha et al., 2001; Yilmaz and Toledo, 2006; Fiori, 2007; Freitas, 2007; Prasain et al., 2009). It also presents antimicrobial activity (Palma et al., 1999). Therefore, this product is commercialized as a food and for cosmetic and pharmaceutical applications (Fiori, 2007).

In the conventional extraction process, the seeds are pressed and then extracted with *n*-hexane (Sovová et al., 1994; Reverchon and De Marco, 2006; Fiori, 2007). The recovery of grape seed oil by SFE has been studied by several researchers as an alternative process, largely because of *n*-hexane's high flammability and hazardous effects to human health (Sovová et al., 1994; Molero Gómez et al., 1996; Palma et al., 1999; Reverchon and Marrone, 2001; Sovová et al., 2001; Cao and Ito, 2003; Bravi et al., 2007; Fiori, 2007, 2010; Freitas, 2007; Passos et al., 2009). Phase equilibrium data for grape seed oil + CO_2 + ethanol were recently reported (Dalmolin et al., 2010).

Molero Gómez et al. (1996) and Cao and Ito (2003) determined global yield isotherms of grape seed oil by SFE at 283–333 K/5–35 MPa and 308–318 K/20–30 MPa, respectively. Molero Gómez et al. (1996) found the maximum yield at 313 K/35 MPa, and 313 K was determined to be the best extraction temperature. It

* Corresponding author. Tel.: +55 19 35214033; fax: +55 19 35214027.
E-mail address: meireles@fea.unicamp.br (M.A.A. Meireles).

0260-8774
doi:10.1016/j.jfoodeng.2011.10.007

was also observed that there is no difference in chemical composition between the oil extracted by Soxhlet with *n*-hexane and by SFE (Molero Gómez et al., 1996; Cao and Ito, 2003).

In addition to temperature, pressure is a parameter that exerts substantial influence on grape seed oil yield; yield increases as pressure increases (Molero Gómez et al., 1996; Cao and Ito, 2003; Fiori, 2007). Several authors have also determined the influence of particle size on the oil yield (Sovová et al., 1994; Molero Gómez et al., 1996; Reverchon and Marrone, 2001; Cao and Ito, 2003; Fiori, 2007), concluding that the smaller the particle size, the higher the total extractable oil by SFE; however, the extraction rate is not altered by this parameter in the initial stage of extraction (Reverchon and Marrone, 2001; Fiori, 2007).

Freitas (2007) compared the yield and oil composition obtained from several grape seed varieties by different methods (pressing, Soxhlet, ultrasound assisted extraction – UAE, pressurized liquid extraction – PLE, SFE). Extraction yields from pressing varied between 1.35% and 6.66%, while Soxhlet yields were between 7.4% and 13.4%. UAE resulted in 8.77–10.6% yields, and PLE yielded 7.0–18.3% yields. For the SFE process with supercritical CO_2, the yields were between 0.56% and 7.9%; with propane, the yield increased to 10.8%. SFE using modifiers (ethanol, *n*-hexane, dichloromethane, ethyl acetate and methanol) was also tested, increasing the yield to 7.8–11.4%. However, although higher yields were achieved using co-solvents, some of them are toxic, which should be avoided to maintain the SFE green label. The SFE process proved to be more selective for vitamin E extraction.

Considering the increasing consumer demand for "natural" products and the stricter government regulations on the use of organic solvents such as hexane, the future of supercritical CO_2 processing of lipids is bright (Temelli, 2009). However, there is still no industrial plant working with SFE in Latin America, and thus, technical and economic evaluation relevant to Brazilian reality is important to provide information for the installation of an industrial SFE unit in that country. Therefore, the objective of this work was to further develop the study of SFE scale-up within Brazilian realities. In this context, grape seed was the raw material selected for validating the scale-up study.

2. Materials and methods

2.1. Raw material characterization

Grape seeds of Malbec and Cabernet Franc varieties (1:1) were donated by the Villa Francioni winery (São Joaquim, SC, Brazil). The seeds were collected in April 2009, after wine fermentation, and were separated from stalks and peels by sieving and air blowing. Some of the peels could not be separated from the seeds by these methods and remained mixed with the seeds. The resulting sample was dried under the sun for 7 days. From a total wet sample of 260 kg, 40 kg of dry seeds were obtained. They were then packed in dark plastic bags and transported to the laboratory.

The seeds were frozen at 255 K and then comminuted in a knife mill (Marconi, model MA 340, Piracicaba, SP, Brazil). The ground raw material was classified according to particle size using a vibratory system (Bertel, model 1868, Caieiras, SP, Brazil) with 8–80 mesh sieves (Tyler series, Wheeling, IL, USA) and then stored in a domestic freezer at 255 K prior to extraction. The mean particle diameter (d_p) was determined according to ASAE Standards (ASAE, 1998).

The moisture content of the raw material was determined in duplicate by xylene distillation (Jacobs, 1973). The true density of the particles (ρ_t) was determined by picnometry with helium gas (Micrometrics, model Multivolume Pycnometer 1305, Norcross, GA, USA) at Central Analítica of the Institute of Chemistry/UNICAMP (Campinas, SP, Brazil). The bed apparent density (ρ_a) was calculated by dividing the feed mass by the vessel volume. The total porosity of the bed and particles was calculated as $(1 - \rho_a/\rho_t)$.

2.2. Overall extraction curve determination

Laboratory-scale SFE equipment (Applied Separations, model 7071, Allentown, PA, USA) equipped with a 0.29 L extraction vessel was used to determine the overall extraction curve (OEC) of grape seed at the laboratory scale (LS). The solvent used was carbon dioxide (99.9% purity, Gama Gases, São Bernardo do Campo, SP, Brazil). This OEC was determined in duplicate and was used as a reference for scaling up the process to the pilot scale.

The extraction conditions selected were 313 K/35 MPa, as determined from data in the literature concerning SFE of grape seed oil (Molero Gómez et al., 1996; Reverchon and Marrone, 2001; Fiori, 2007). The separator consisted of a 0.05 L glass vial immersed in an ice bath at ambient pressure. All experimental data are presented in Table 1.

The OEC obtained was fitted to two straight lines, namely, the CER (constant extraction rate) and DC (diffusion controlled) periods, according to the method described by Rodrigues et al. (2002) and Meireles (2008).

2.3. Scale-up and separation step study

The scale-up criterion adopted consisted of maintaining a constant solvent mass to feed mass ratio (S/F), as recommended by Prado et al. (2011). The LS OEC was used as a reference for determining the pilot-scale (PS) OEC; the operational conditions were the same except for the solvent flow rate, which was calculated using the S/F constant scale-up criterion so that a S/F of 8.2 for 300 min of the process was maintained from LS to PS (Table 1).To analyze the extraction curves as a function of time, another parameter was kept constant: Q_{CO_2}/F of $4.5 \times 10^{-4}\ s^{-1}$. The combination of keeping $S/F + Q_{CO_2}/F$ constant was suggested by Mezzomo et al. (2009) as an efficient scale-up criterion; however, Prado et al. (2011) used a different Q_{CO_2}/F for the same S/F and still obtained similar extraction curves. Therefore, S/F is considered the effective scale-up criterion.

Pilot-scale equipment (Thar Technologies, model SFE-2×5LF-2-FMC, Pittsburgh, PA, USA) equipped with two 5.15 L extraction vessels and three 1 L separators in series was used for scale-up experiments; only one extractor was used. The solvent used was carbon dioxide (99.0% purity, Gama Gases, São Bernardo do Campo, SP, Brazil). All experiments were conducted in duplicate.

In contrast to bench equipment, in the pilot equipment, it is not possible to collect the extracts continuously during the kinetics experiments. It is necessary to interrupt the solvent flow and then depressurize the separators while keeping the extractor pressurized so that the extract can be collected; after this procedure, the extraction can be resumed until the next collection point. Interrupting the extraction at each point implies intermediary static periods in the extractor. To determine their influence on the yield, a second type of large-scale experiment was conducted: total collection. The difference from the kinetics experiment is that the extract was collected only at the end of the experiment, without intermediary interruptions (Table 1).

2.4. Chemical composition of the extracts

The fatty acid composition of the extracts was determined in duplicate by gas chromatography, according to the official method Ce 1–62 of the AOCS (1997). Prior to chromatographic analysis, the fatty samples were prepared in the form of fatty acid methyl esters (FAME) according to the method of Hartman and Lago (1973). The chromatographic analyses were performed using a GC capillary gas

Table 1
Bed characterization and operational data of experiments.

	Lab scale (LS)	Pilot scale (PS)	Total collection (TC)
Raw material characterization			
Moisture (%) [a]	12 ± 1	12 ± 1	12 ± 1
d_p (10^{-4} m)[c]	7.79	7.79	7.79
ρ_t (kg/m^3) [b,d]	1408 ± 25	1408 ± 25	1408 ± 25
Experimental data			
F (g) [a,e]	280 ± 5	4677 ± 69	4890 ± 3
ρ_a (kg/m^3) [a,f]	966 ± 18	908 ± 13	949 ± 1
Porosity	0.32 ± 0.02	0.36 ± 0.02	0.33 ± 0.02
H_b/d_b[g]	2.31	5.94	5.94
T in extractor (K)[h]	313	313	313
P in extractor (MPa)[i]	35	35	35
T in S_1 (K)[h,j]	278	313	313
P in S_1 (MPa)[i,j]	0.1	10	10
T in S_2 (K)[h,k]	–	313	313
P in S_2 (MPa)[i,k]	–	6	6
T in S_3 (K)[h,l]	–	313	313
P in S_3 (MPa)[i,l]	–	3	3
Static period (min)	20	20	20
Q_{CO_2} (10^{-3} kg/s) [a,m]	0.128 ± 0.003	2.14 ± 0.02	2.16 ± 0.01
S/F total (kg CO_2/kg grape seed) [a,n]	12.6 ± 0.3	8.21 ± 0.01	8.23 ± 0.01
S/F in 300 min (kg CO_2/kg grape seed) [a,n]	8.4 ± 0.3	8.21 ± 0.01	8.23 ± 0.01
Total time (min)	450	300	300
t_{RES} (min)[o]	11	11	10
v (m/s)[p]	0.06	0.25	0.26

[a] Values presented with amplitude of two determinations.
[b] Values presented with standard deviation of 10 repetitions.
[c] Mean particle diameter (d_p).
[d] True density of particles (ρ_t).
[e] Raw material mass (F).
[f] Bed apparent density (ρ_a).
[g] Bed height to diameter ratio (H_b/d_b).
[h] Temperature (T).
[i] Pressure (P).
[j] Separator 1 (S_1).
[k] Separator 2 (S_2).
[l] Separator 3 (S_3).
[m] CO_2 flow rate(Q_{CO2}).
[n] Solvent to feed ratio (S/F).
[o] Solvent residence time (t_{RES}).
[p] Solvent superficial velocity (v).

chromatograph system (Agilent, 6850 Series GC System, Santa Clara, CA, USA) under the following experimental conditions: DB-23 capillary column (Agilent, 50% cyanopropyl-methylpolysiloxane, 0.25 μm × 60 m × 0.25 mm i.d., Santa Clara, CA, USA); helium as carrier gas at a flow rate of 0.001 L/min; linear velocity of 24 cm/s; injection temperature of 523 K; column temperature of 383 K for 5 min, (383–488) K (rate of 5 K/min), 488 K for 34 min; detection temperature of 553 K; and injection volume of 1.0 μL. The fatty acid methyl esters were identified by comparison with external standards purchased from Nu Check Prep (Elysian, MN, USA). Quantification was performed by internal normalization. These analyses were performed at the Laboratório de Óleos e Gorduras of the School of Food Engineering/UNICAMP (Campinas, SP, Brazil).

The acylglycerol and free fatty acid compositions of the extracts recovered in tetrahydrofuran (THF) were analyzed by high-performance size-exclusion (HPSEC) in a PerkinElmer 250 liquid chromatograph (Waltham, MA, USA) with a refractive index detector (Sicon Analytic) and with one 100 Å and one 500 Å JORDI GEL DVB column (300 mm × 7.8 mm) connected in series. The columns were 300 × 7.8 mm i.d., and THF served as the mobile phase with a flow of 1 mL/min. This analysis was performed at the Laboratório de Óleos e Gorduras of the School of Food Engineering/UNICAMP (Campinas, SP, Brazil).

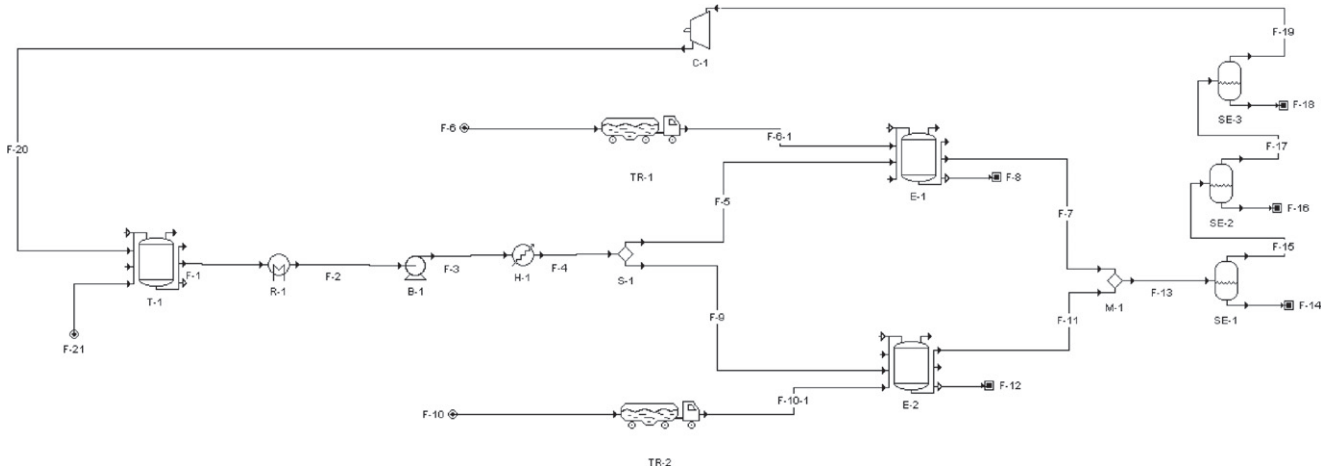
2.5. Differential scanning calorimetry (DSC)

The crystallization and melting temperature of crude grape seed oil was characterized by a differential scanning calorimeter (DSC) equipment (MDSC 2920 model, TA Instruments, New Castle, DE, USA) with a refrigerated cooling system that, in this study, operated between 204 K and 320 K. The DSC was calibrated for temperature and heat flow using indium (99.999%) certified by TA Instruments and with cyclohexane (min 99.99%) and naphthalene (min 99%), both from Merck.

Samples (2–5 mg) were weighed in a microanalytical balance (AD6 model, PerkinElmer, Norwalk, CT, USA) with an accuracy of $\pm 0.2 \times 10^{-5}$ g and were placed in sealed aluminum pans. An empty pan was used as a reference in the DSC equipment. The samples were analyzed during a cooling and a heating program. First, the samples were cooled from 320 K to 204 K at a cooling rate of 1 K/min. After an isothermal period of 5 min at 204 K, the samples were heated to 320 K at a heating rate of 1 K/min. Peak top temperatures were measured for crude grape seed oil using the analysis software from TA instruments. Peak top temperatures were also considered to determine the temperature of the phase transitions associated with overlapped peaks. The analyses were performed in triplicate.

2.6. Economic evaluation

The commercial simulator SuperPro Designer v6.0 was used to estimate the cost of manufacturing (COM) of grape seed extract. The methodology developed by Prado et al. (2009) was adapted (Fig. 1). The economic data fed to the simulator are presented on Table 2. Three extraction units of different scales were evaluated, all with the same design (Fig. 1), which was based on the pilot



Streams

F-1/F-2/F-3/F-4/F-5/F-9 – CO_2 feeding
F-6/F-6-1/F-10/F-10-1 – raw material feeding
F-7/F-11/F-13 – CO_2 + extract exit
F-8/F-12 – solid residue exit + CO_2 loss
F-14/F-16/F-18 – product exits
F-15/F-17 – extract and CO_2 fractionation
F-19/F-20 – CO_2 recyclying
F-21 – replacement of CO_2 lost

Equipments

B-1 – CO_2 pump
C-1 – compressor
E-1/E-2 – extractors
H-1 – heat exchanger for CO_2 heating
M-1/S-1 – stream mixer and separator, respectively
R-1 – heat exchanger for CO_2 cooling
SE-1/SE-2/SE-3 – separators
T-1 – CO_2 tank
TR-1/TR-2 – raw material pre-processing

Fig. 1. Scheme of SFE process built in SuperPro Designer simulator, used for economical evaluation.

Table 2
Economic parameters used for COM estimation.

Industrial units[a]	
2 extractors of 5 L	US$ 100,000.00
2 extractors of 50 L	US$ 300,000.00
2 extractors of 500 L	US$ 1,150,000.00
Depreciation rate	10%/year
Labor[a]	US$ 4.00/h
2 extractors of 5 L	1 operator
2 extractors of 50 L	2 operators
2 extractors of 500 L	3 operators
Raw materials	
Wet grape seed	0 and US$ 2.70/ton
Pre-processing	US$ 40.00/ton
CO_2 (2% loss)	US$ 0.15/kg
Utilities[a]	
Electricity	US$ 0.092/kWh
Cooling water	US$ 0.19/ton
Steam	US$ 4.20/ton

[a] Prado (2009).

equipment but with extractor volumes ranging from 5 to 500 L (Table 2). Operational data were based on the pilot-scale (PS) experiment, and the scale-up criterion for 50 and 500 L consisted of keeping S/F constant.

For purposes of the estimate, the industrial unit will run 24 h with three daily shifts for 330 days, which represents a total of 7920 h of operation per year; 30 days will be reserved for plant maintenance (Rosa and Meireles, 2005). The number of operators needed per shift varies according to the capacity of the plant (Table 2). Labor charges and labor not directly associated with production were estimated by the simulator.

The raw material cost is related to the plant material and CO_2 lost during the process. CO_2 loss is mainly due to depressurization of the extractor at the end of each batch (Perrut, 2007). Wet grape seed can be sold for up to US$ 2.70/ton in Brazil, but in some cases, it is donated by wineries in return for residue transportation. COM was estimated considering costs of zero and US$ 2.70/ton of wet grape seed. Pre-processing costs involve drying and comminution of raw material.

Utility costs comprise producing heat exchange agents and the electricity used in the process. Utilities needed for the operation of each type of equipment were estimated by the simulator energy balance. The cost of waste treatment may be neglected because the residue of the SFE process is a dry solid vegetable matrix that may be incorporated into the soil or commercialized as a by-product, as it does not contain any residue of toxic solvents. The CO_2 lost during system depressurization needs no treatment because in small quantities, it is not toxic. Transportation costs still need to be added to the estimated COM.

3. Results and discussion

3.1. Scale-up study

Fig. 2 shows the OECs obtained for grape seed SFE at the laboratory (LS) and pilot (PS) scales. LS OEC presents a long CER (constant extraction rate) period of 314 min, which accounts for almost 90% of the total extract. A DC (diffusion controlled) period follows, with a rapid decrease in the extraction rate; an intermediary FER (falling extraction rate) period cannot be observed. The kinetic parameters calculated from the adjustment of the LSOEC were t_{CER} = 314 min, $M_{CER} = 1.78 \times 10^{-6}$ kg/s, $Y_{CER} = 1.42 \times 10^{-2}$ kg extract/kg CO_2 and R_{CER} = 12.02% (dry basis, d.b.), where t_{CER} is

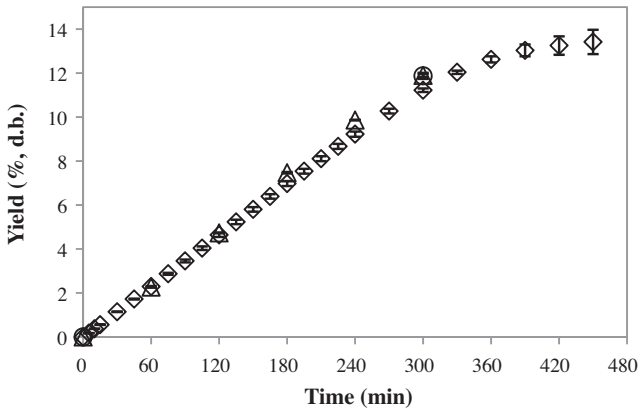


Fig. 2. OECs of grape seed SFE at 313 K/35 MPa for laboratory (◇) and pilot (△) scales, and total collection yield at pilot scale (○).

the time of the CER, while M_{CER} is the mass-transfer rate and R_{CER} is the yield achieved during this period. The value of t_{CER} roughly represents the minimum time a SFE cycle should last to keep the process economically viable (Meireles, 2008). Y_{CER} is the mass ratio of solute in the supercritical phase at the bed outlet during the CER period.

The total yield in 450 min of extraction was 13.42% (d.b.). This result is consistent with the data in the literature, which report grape seed oil yields obtained by SFE ranging from 2.0% to 12.5% (Sovová et al., 1994; Molero Gómez et al., 1996; Reverchon and Marrone, 2001; Fiori, 2007; Freitas, 2007). In 450 min, S/F = 12.6 was used; this amount of solvent was enough to exhaust the extraction bed and is in accordance with the data in the literature (Fiori, 2007).

Studying scale-up is important to establish a methodology that makes it possible to predict the behavior of the SFE process at an industrial scale from laboratory-scale data. The relationships between processes conducted at the bench, pilot and industrial scales cannot always be simply approached or predicted. Therefore, the differences observed in procedures conducted in equipment of significantly different sizes must be carefully studied and evaluated to avoid serious mistakes when scaling-up a process.

A 17-fold scale-up from bench to pilot scale was achieved for grape seed. The similar shapes of the OECs reveal that the simple criterion used (constant S/F) was successful in reproducing kinetic behavior at different scales. It can also be noted that the PS OEC indicated a slightly higher yield than the LS OEC [6% higher at 300 min, (11.90 ± 0.07)% for PS than for LS (11.22 ± 0.08)%]. This result is in agreement with other scale-up studies conducted by our research group (Prado et al., 2011; Prado and Meireles, 2010).

The open data in the literature on the scale-up of SFE are different from equipment manufacturers' information. While the latter claim that the SFE process is more efficient at larger scales, studies in the literature report lower yields as the operation scale increases (Del Valle et al., 2004; Kotnik et al., 2007). The results obtained in the present study are, therefore, in agreement with the equipment manufacturers' information. The fact that the LS OEC shape was successfully reproduced in the PS experiment indicates that S/F can be used as an efficient prediction criterion of SFE behavior as scale increases. However, some other parameter, as discussed below, also influences the process so that the yield increases with scale-up. Even though the curves were not exactly reproduced, the results found are important because this criterion makes it possible to simplify the SFE scale-up study using only a few laboratory data and simple calculation to estimate the minimum yield expected as scale increases to the industrial level. Considering the expectation of more efficient processes at the industrial scale, the simple scale-up criterion adopted in the present work is more efficient for predicting the extraction behavior as scale increases than some of the more complex models presented in the literature.

Several factors could have influenced the higher yields obtained for the pilot-scale experiments, such as the intermediary static periods, the higher solvent superficial velocity causing mechanical dragging (entrainment of oil not solubilized by the CO_2 flow) and the efficiency of the separators in recovering the extract.

A total collection (TC) experiment was conducted to determine the influence of the intermediary static periods on the yield when determining an OEC at the pilot scale. In Fig. 2, it can be noted that there is no difference between the PS (11.90%) and TC (11.88%) yields at the pilot scale, indicating that the procedure of using intermediary static periods at the pilot scale does not influence the extraction yield. Therefore, the methodology adapted for determining OECs at the pilot scale can be used for comparing data to lab-scale OECs.

Prado et al. (2011) presented a discussion on how the mechanical dragging and efficiency of the separators can influence the process. With increasing solvent mass flow, increasing amounts of the condensed phase are entrained (Brunner, 1994). Prado et al. (2011) tested a 2.5-fold increase in solvent superficial velocity using the same pilot-scale equipment used in the present work to assess the influence of this parameter. Despite some indication that the increase in superficial velocity does influence the process, the study was inconclusive. Therefore, a deeper study of these factors is necessary to determine which of these factors, and in which magnitude, influence the SFE process in the pilot equipment so that the yields are higher than the yields found for the bench scale.

Del Valle et al. (2004) suggested that a simple criterion should be considered with caution because of the several phenomena involved in the SFE process, which could only be covered by complex models. On the other hand, the simple criterion adopted proved to be more efficient than many complex models proposed in the literature for SFE scale-up, and the higher yields achieved in the present study were in agreement with the equipment manufacturers' information. Therefore, maintaining a constant S/F proportion is a consistent scale-up criterion for predicting SFE behavior at different scales, which could be easily used for industrial applications.

3.2. Separation step study

Fig. 3 presents the yields obtained in each separator at the pilot scale. S_1 (separator 1), S_2 (separator 2) and S_3 (separator 3) worked at 313 K/10 MPa, 313 K/6 MPa and 313 K/3 MPa, respectively. Most

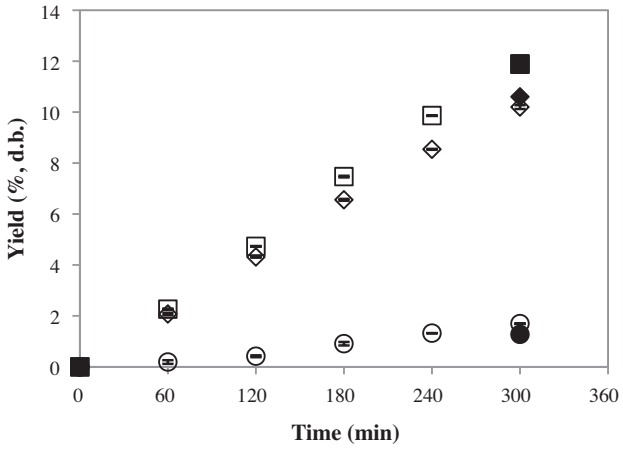


Fig. 3. Yields of the separators for grape seed SFE at 313 K/35 MPa at pilot scale: S_1 (◇), S_2 (○) and total yield (□). Open and filled symbols represent PS and TC experiments, respectively.

of the extract was recovered in S_1 (86%), and the rest of it was recovered in S_2. There was no extract in S_3, which indicates that using the operational conditions selected for S_2, it was possible to precipitate all the extract. It is important to determine the minimum pressure to be used in the separators, as the cost of recompressing the solvent for recycling decreases with increased pressure (Takeuchi et al., 2008). Therefore, using 313 K/6 MPa as the operation conditions of an industrial separator may be economically advantageous.

Observing Fig. 3, it is once again possible to notice that the TC experiment was compatible with the PS experiment, which indicates the efficiency of adapting the method of determining OEC at the pilot scale, with the interruption of the solvent flow at each point.

Regarding the physical aspects of the products, the extracts obtained in S_1 changed from an opaque yellowish-green color at the beginning of extraction to dark green at the end; the extracts presented a liquid consistency. The extracts recovered in S_2 presented a whitish-yellow color and a pasty consistency. The grape seed oil extracted by conventional process presents a light green translucent color after the refining process.

3.3. Chemical composition of the extracts

Table 3 presents the chemical composition of the grape seed oil obtained by SFE compared with the Codex Alimentarius identity specifications (Codex Stan 210, 2009). The fractions of the PS experiment collected in S_1 and S_2 were analyzed individually, whereas for the TC experiment, the S_1 and S_2 fractions were mixed before analysis.

Crude grape seed oil consists mainly of linoleic (cis-9,*cis*-12-octadecadienoic) and oleic(*cis*-9-octadecenoic) unsaturated fatty acids and also of palmitic (Hexadecanoic) and stearic (Octadecanoic) saturated fatty acids. At the beginning of extraction, around 98% of the fatty acids are recovered in the form of triacylglycerols and diacylglycerols in S_1, but in S_2, there is only about 72% of triacylglycerols and diacylglycerols, with a higher concentration of free fatty acids and monoacylglycerols. This fractionation of the acylglycerol mixtures was affected by both solubility in CO_2 and the vapor pressure of the components when subjected to different pressures in separators. At the end of the extraction, there was a higher concentration of triacylglycerols and diacylglycerols in S_1. Recently, Dalmolin et al. (2010) studied the chemical characterization of the extracts obtained by supercritical CO_2 extraction of grape seeds. These authors showed that the content of *trans*-resveratrol in grape seed extracts was similar to the contents reported in the literature for red wines, demonstrating that a considerable amount of *trans*-resveratrol remains in grape seeds after the fermentation process in wine production and thus showing that the SFE process may be useful for recovering bioactive compounds present in the seed. It can also be noted that the chemical

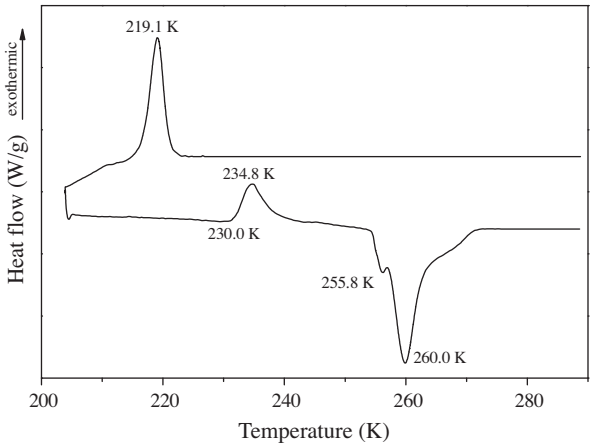


Fig. 4. Thermogram of crude grape seed oil. The samples were cooled from 320 K to 204 K at a cooling rate of 1 K/min. An isothermal period of 5 min at 204 K was used, then, the samples were heated to 320 K at a heating rate of 1 K/min.

Table 3
Chemical composition of grape seed oil obtained by supercritical fluid extraction.

	PS[a]					Codex Alimentarius
	S_1[b] (60 min)	S_2[c] (60 min)	S_1[b] (300 min)	S_2[c] (300 min)	TC[d]	
HPSEC (%)						
Triacylglycerols + diacylglycerols	97.90	72.14	98.98	97.17	98.27	
Monoacylglycerols + free fatty acids	2.10	27.86	1.02	2.83	1.73	
Fatty acid composition (%, w/w)						
Octanoic C (8:0)		0.23		0.03		ND[e]
Decanoic C (10:0)	0.03	0.71		0.03	0.02	ND
Dodecanoic C (12:0)	0.04	0.74		0.04	0.03	ND
Tetradecanoic C (14:0)	0.14	0.69	0.08	0.12	0.11	<0.30
Pentadecanoic C (15:0)	0.03	0.10		0.04	0.02	
Hexadecanoic C (16:0)	8.57	13.32	7.50	8.56	8.13	5.5–11.00
cis-9-hexadecanoic C (16:1)	0.19	0.57	0.13	0.18	0.15	<1.2
Heptadecanoic C (17:0)	0.07	0.11	0.07	0.07	0.07	<0.2
cis-heptadec-9-enoic C (17:1)	0.02		0.02	0.03	0.03	<0.1
Octadecanoic C (18:0)	3.81	4.21	4.45	4.07	4.05	3.0–6.5
cis-9-octadecenoic C (18:1)	14.75	13.55	15.63	15.28	15.10	12.0–28.0
cis-9,*cis*-12-octadecadienoic C (18:2)	71.23	63.25	70.95	70.39	71.20	58.0–78.0
All-*cis*-9,12,15-octadecatrienoic C (18:3)	0.62	1.67	0.52	0.58	0.57	<1.0
Eicosanoic C (20:0)	0.20	0.26	0.28	0.23	0.22	<1.0
cis-9-eicosanoic C (20:1)	0.15	0.15	0.22	0.16	0.18	<0.3
Docosanoic C (22:0)	0.09	0.26	0.08	0.11	0.07	<0.5
Tetracosanoic C (24:0)	0.06	0.18	0.07	0.08	0.05	<0.4

[a] Pilot scale.
[b] Separator 1.
[c] Separator 2.
[d] Total collection.
[e] non-detectable, defined as <0.05%.

compositions of the oil samples used in this work are all within the Codex Alimentarius identity specifications, which certify that the oil was derived from the seeds of grape (*Vitis vinifera* L.).

3.4. Differential scanning calorimetry (DSC)

The DSC result for the extract is presented in Fig. 4. It presented a crystallization peak at 219.1 K with an enthalpy of 29.7 J/g. Polymorphism was detected in the extract. After melting around 230.0 K, an exothermic peak (234.8 K) was found related to an additional crystallization. The extract presented a transition at 255.8 K, associated with a polymorphic change. At 260.0 K, another peak associated with the melting of the extract appears, with an enthalpy of 65.0 J/g. Similar DSC tracings were observed for grape seed oil by Kaisersberger (1990); the first endothermic peak (230.0 K) represents the melting of the unstable α crystal form followed by the crystallization of the more stable β form, which is characterized by an exothermic peak.

Table 4
Economic evaluation of grape seed SFE with cost zero for raw material.

Time (min)	Productivity (kg/year)			Operation cost (US$/year)	COM[a] (US$/kg extract)	CRM[b] (%)	COL[c] (%)	FCI[d] (%)	CQC[e] (%)	CUT[f] (%)	Return (years)
	S_1	S_2	Total								
5 L											
60	382	35	417	121,000	290.17	0.98	60.32	29.06	9.05	0.59	NV[g]
120	503	49	552	120,000	217.39	0.67	60.47	29.12	9.07	0.67	NV
180	562	78	640	120,000	187.50	0.53	60.53	29.15	9.08	0.70	NV
240	577	90	667	120,000	179.91	0.42	60.57	29.20	9.09	0.73	NV
300	569	95	664	120,000	180.72	0.40	60.57	29.20	9.09	0.74	NV
50 L											
60	3815	349	4164	292,000	70.12	4.06	49.93	36.09	7.49	2.43	NV
120	5032	490	5522	289,000	52.34	2.80	50.43	36.43	7.56	2.78	NV
180	5616	779	6395	288,000	45.04	2.23	50.64	36.59	7.60	2.94	46.93
240	5773	899	6672	287,000	43.02	1.77	50.82	36.75	7.62	3.04	21.88
300	5690	947	6637	286,000	43.09	1.68	50.83	36.76	7.62	3.10	23.18
500 L											
60	38,153	3485	41,638	844,000	20.27	14.02	25.88	47.80	3.88	8.41	2.92
120	50,319	4904	55,223	816,000	14.78	9.90	26.78	49.45	4.02	9.85	1.92
180	56,160	7789	63,949	804,000	12.57	7.98	27.19	50.20	4.08	10.54	1.58
240	57,731	8995	66,726	793,000	11.88	2.57	27.56	50.92	4.13	11.00	1.49
300	56,903	9472	66,375	792,000	11.93	6.09	27.58	50.97	4.14	11.22	1.50

[a] Cost of manufacturing.
[b] Cost of raw material.
[c] Cost of labor.
[d] Fixed cost of investment.
[e] Cost of quality control.
[f] Cost of utilities.
[g] Nonviable.

Table 5
Economic evaluation of grape seed SFE with raw material cost of US$ 2.70/ton.

Time (min)	Productivity (kg/year)			Operation cost (US$/year)	COM[a] (US$/kg extract)	CRM[b] (%)	COL[c] (%)	FCI[d] (%)	CQC[e] (%)	CUT[f] (%)	Return (years)
	S1	S2	Total								
5 L											
60	382	35	417	121,000	290.17	1.28	60.14	28.98	9.02	0.59	NV[g]
120	503	49	552	121,000	219.20	0.86	60.35	29.07	9.05	0.67	NV
180	562	78	640	121,000	189.06	0.67	60.45	29.11	9.07	0.70	NV
240	577	90	667	120,000	179.91	0.53	60.50	29.16	9.08	0.72	NV
300	569	95	664	120,000	180.72	0.49	60.52	29.18	9.08	0.74	NV
50 L											
60	3815	349	4164	295,000	70.85	5.23	49.32	35.65	7.40	2.40	NV
120	5032	490	5522	291,000	52.70	3.57	50.03	36.14	7.50	2.76	NV
180	5616	779	6395	289,000	45.19	2.80	50.35	36.37	7.55	2.93	55.62
240	5773	899	6672	288,000	43.17	2.22	50.59	36.58	7.59	3.03	23.22
300	5690	947	6637	287,000	43.24	2.06	50.64	36.62	7.60	3.09	24.41
500 L											
60	38,153	3485	41,638	880,000	21.13	17.54	24.82	45.85	3.72	8.06	3.03
120	50,319	4904	55,223	839,000	15.19	12.36	26.05	48.10	3.91	9.58	1.95
180	56,160	7789	63,949	821,000	12.84	9.87	26.64	49.17	4.00	10.33	1.59
240	57,731	8995	66,726	806,000	12.08	7.92	27.10	50.08	4.07	10.82	1.50
300	56,903	9472	66,375	803,000	12.10	7.37	27.20	50.28	4.08	11.06	1.51

[a] Cost of manufacturing.
[b] Cost of raw material.
[c] Cost of labor.
[d] Fixed cost of investment.
[e] Cost of quality control.
[f] Cost of utilities.
[g] Nonviable.

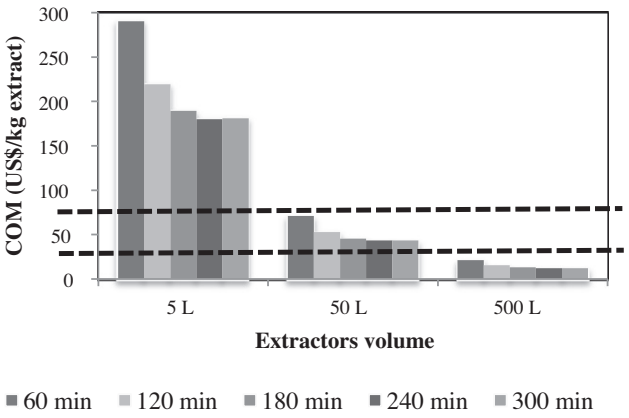


Fig. 5. COM estimation of grape seed oil obtained by SFE with grape seed cost of US$ 2.70/ton; dotted lines represent selling price of oil obtained by cold pressing in Brazilian (US$ 40,00) and international (US$ 80,00) markets.

3.5. Economic evaluation

Two scenarios found in the grape seed market were evaluated: a zero cost of raw material (Table 4) and a cost of wet raw material of US$ 2.70/ton (Table 5, Fig. 5). The dotted lines in Fig. 5 represent the selling price of grape seed oil obtained by cold pressing, in both Brazilian and international markets. The price of grape seed oil obtained by *n*-hexane extraction is US$ 4.85/kg (Liberty Natural, 2010); however, the product closest to SFE oil is the pressed one, because they both present no traces of toxic solvents. The return time was calculated considering the lower price found in the market (Liberty Natural, 2010) for pressed oil (US$ 40.00/kg).

Because it is a raw material with low added value, the grape seed price presented little influence on COM, with a maximum difference of approximately US$ 2.00/kg of product between the process using donated raw material (Table 4) and the process using purchased raw material (Table 5). The SFE process starts being economically viable in the 50 L plant, depending on the selling price of the product (Fig. 5). For a selling price of US$ 40.00/kg, only the 500 L plant is viable, with a minimum COM of US$ 12.00/kg and return time of 1.5 years. Fiori (2010) estimated the break-even point for a return time of 10 years for SFE of grape seed oil with three extractors in series working in counter-current at 5.9 €/kg, that is, within the values estimated in this work. Both options seem viable from an economic point of view.

The major cost share moves from COL to FCI as plant capacity increases. CRM presents a low share, mainly because of CO_2 loss in the depressurization step.

From yield and economic data, it may be concluded that for SFE of grape seed, 313 K/35 MPa, 240 min and S/F of 6.6 offer the best relationship between cost and oil yield. Still, it should be remembered that because of the different physical–chemical aspects presented by the extracts recovered in the different separators, the selling price of the products may vary.

On the basis of the scale-up data determined, the yield increases with increasing scale. This information is extremely relevant from economic evaluation point of view, because it means that with further scale increases for industrial application, the COM can be further reduced.

4. Conclusions

The simple criterion adopted in this study (constant S/F ratio) consistently reproduces OECs at different scales, with slightly higher yields as scale increases. This scale-up procedure makes it possible to simplify the study of the SFE process by allowing prediction of the behavior of the process at the industrial scale from laboratory data with no need for complex calculations. Thus, laboratory-scale data can be safely used to develop economic evaluations of the SFE process, as the tendency of the process is to improve yield with increasing scale.

The grape seed oil obtained by SFE in this work presents a chemical profile within identity specifications. In terms of product quality and environmental issues, SFE can be regarded as a suitable alternative for processing heat-sensitive materials, providing appropriate conditions to maintain the functional properties of grape seed oil.

The estimation of manufacturing cost showed that it is economically viable to establish a SFE plant in Brazil for processing grape seed. From the technical-economic evaluation, for SFE of grape seed at 313 K/35 MPa, an extraction time of 240 min and S/F of 6.6 presented the best relationship between yield and cost.

Acknowledgements

The authors thank the Villa Francioni winery (São Joaquim, SC) for raw material donation, FAPESP (2007/03817-7, 2008/56258-8) and CNPq for PhD assistantships, and CAPES/PROCAD (840/2008) for financial support.

References

AOCS – American Oil Chemists' Society, 1997. Official Methods and Recommended Practices of the American Oil Chemists' Society, fifth ed., vol. 2. AOCS Press, Champaign, pp. 1–4 (Ce 1–62).

ASAE – American Society of Agricultural Engineers, 1998. Method of Determining and Expressing Fineness of Feed Materials by Sieving. In: American Society of Agricultural Engineers Standards, S319.3. American Society of Agricultural & Biological Engineers, St. Joseph, pp. 447–550.

Bravi, M., Spinoglio, F., Verdone, N., Adami, M., Aliboni, A., d'Andrea, A., de Santis, A., Ferri, D., 2007. Improving the extraction of α-tocopherol-enriched oil from grape seeds by supercritical CO_2. Optimization of the extraction conditions. Journal of Food Engineering 78 (2), 488–493.

Brunner, G., 1994. Gas Extraction: An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes, first ed. Steinkopff, Darmstadt, pp. 175–178.

Cao, X., Ito, Y., 2003. Supercritical fluid extraction of grape seed oil and subsequent separation of free fatty acids by high-speed counter-current chromatography. Journal of Chromatography A 1021 (1), 117–124.

Carvalho Jr., R.N., Moura, L.S., Rosa, P.T.V., Meireles, M.A.A., 2005. Supercritical fluid extraction from rosemary (*Rosmarinus officinalis*): kinetic data, extract's global yield, composition and antioxidant activity. Journal of Supercritical Fluids 35 (3), 197–204.

Codex Stan 210, 2009. Codex Standard for Named Vegetable Oils. Codex-Stan 210-1999, pp. 1–13.

Dalmolin, I., Mazutti, M.A., Batista, E.A.C., Meireles, M.A.A., Oliveira, J.V., 2010. Chemical characterization and phase behavior of grape seed oil in compressed carbon dioxide and ethanol as co-solvent. Journal of Chemical Thermodynamics 42 (6), 797–801.

Del Valle, J.M., Rivera, O., Mattea, M., Ruetsch, L., Daghero, J., Flores, A., 2004. Supercritical CO_2 processing of pretreated rosehip seeds: effect of process scale on oil extraction kinetics. Journal of Supercritical Fluids 31 (2), 159–174.

Fiori, L., 2007. Grape seed oil supercritical extraction kinetic and solubility data: critical approach and modeling. Journal of Supercritical Fluids 43 (1), 43–54.

Fiori, L., 2010. Supercritical extraction of grape seed oil at industrial scale: plant process design, modeling, economic feasibility. Chemical Engineering and Processing 49, 866–872.

Freitas, L.S., 2007. Desenvolvimento de procedimentos de extração do óleo de semente de uva e caracterização química dos compostos extraídos. Ph.D. Thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil (in Portuguese).

Han, X., Cheng, L., Zhang, R., Bi, J., 2009. Extraction of safflower seed oil by supercritical CO_2. Journal of Food Engineering 92 (4), 370–376.

Hartman, L., Lago, R.C.A., 1973. Rapid preparation of fatty acid methyl esters from lipids. Laboratory Practice 22, 475–476.

Jacobs, M.B., 1973. The Chemical Analysis of Foods and Food Products, third ed. Publishing Co., New York, pp. 22–23.

Jayaprakasha, G.K., Singh, R.P., Sakariah, K.K., 2001. Antioxidant activity of grape seed (*Vitisvinifera*) extracts on peroxidation models in vitro. Food Chemistry 73 (3), 285–290.

Kaisersberger, E., 1990. Application of heat-flux DSC for the characterisation of edible fats and oils. Analytical Proceedings 27 (3), 64–65.

Kotnik, P., Škerget, M., Knez, Ž., 2007. Supercritical fluid extraction of chamomile flower heads: comparison with conventional extraction, kinetics and scale-up. Journal of Supercritical Fluids 43 (2), 192–198.

Liberty Natural, 2010. <www.libertynatural.com> (accessed October 2010).

Martínez, J., Rosa, P.T.V., Meireles, M.A.A., 2007. Extraction of clove and vetiver oils with supercritical carbon dioxide: modeling and simulation. The Open Chemical Engineering Journal 1 (1), 1–7.

Meireles, M.A.A., 2008. Extraction of bioactive compounds from Latin American plants. In: Martinez, J. (Org.), Supercritical Fluid Extraction of Nutraceuticals and Bioactive Compounds. CRC Press–Taylor and Francis Group, Boca Raton, pp. 243–274.

Mezzomo, N., Martínez, J., Ferreira, S.R.S., 2009. Supercritical fluid extraction of peach (*Prunus persica*) almond oil: kinetics, mathematical modeling and scale-up. Journal of Supercritical Fluids 51 (1), 10–16.

Molero Gómez, A., Pereyra López, C., Martínez de laOssa, E., 1996. Recovery of grape seed oil by liquid and supercritical carbon dioxide extraction: a comparison with conventional solvent extraction. Chemical Engineering Journal 61 (3), 227–231.

Moura, L.S., Carvalho Jr., R.N., Stefanini, M.B., Ming, L.C., Meireles, M.A.A., 2005. Supercritical fluid extraction from fennel (*Foeniculum vulgare*): global yield, composition and kinetic data. Journal of Supercritical Fluids 35 (3), 212–219.

Palma, M., Taylor, L.T., Varela, R.M., Cutler, S.J., Cutler, H.G., 1999. Fractional extraction of compounds from grape seeds by supercritical fluid extraction and analysis for antimicrobial and agrochemical activities. Journal of Agricultural and Food Chemistry 47 (12), 5044–5048.

Passos, C.P., Silva, R.M., Da Silva, F.A., Coimbra, M.A., Silva, C.M., 2009. Enhancement of supercritical fluid extraction of grape seed oil by using enzymatically pre-treated seed. Journal of Supercritical Fluids 48 (3), 225–229.

Perrut, M., 2007. Industrial applications of supercritical fluids: development status and scale-up issues. In: I Iberoamerican Conference on Supercritical Fluids, Foz do Iguaçu, Brazil.

Prado, I.M., 2009. Uso de simulador no estudo de aumento de escala e viabilidade econômica do processo de extração supercrítica de produtos naturais. Master Thesis, Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos, Campinas, Brazil (in Portuguese).

Prado, I.M., Albuquerque, C.L.C., Cavalcanti, R.N., Meireles, M.A.A., 2009. Use of commercial process simulator to estimate the cost of manufacturing (COM) of carotenoids obtained via supercritical technology from palm and buriti trees. In: Ninth International Symposium on Supercritical Fluids, Arcachon, France.

Prado, J.M., Meireles, M.A.A., 2010. Supercritical fluid extraction of lemon verbena (*Aloysia triphylla*): kinetics, scale-up and chemical composition. In: 12th European Meeting on Supercritical Fluids, Graz-Austria.

Prado, J.M., Prado, G.H.C., Meireles, M.A.A., 2011. Scale-up study of supercritical fluid extraction process for clove and sugarcane residue. Journal of Supercritical Fluids 56, 231–237.

Prasain, J.K., Peng, N., Dai, Y., Moore, R., Arabshahi, A., Wilson, L., Barnes, S., Wyss, J.M., Kim, H., Watts, R.L., 2009. Liquid chromatography–tandem mass spectroscopy identification of proanthocyanidins in rat plasma after oral administration of grape seed extract. Phytomedicine 16 (2–3), 233–243.

Reverchon, E., De Marco, I., 2006. Supercritical fluid extraction and fractionation of natural matter. Journal of Supercritical Fluids 38 (2), 146–166.

Reverchon, E., Marrone, C., 1997. Supercritical extraction of clove bud essential oil: isolation and mathematical modeling. Chemical Engineering Science 52 (20), 3421–3428.

Reverchon, E., Marrone, C., 2001. Modeling supercritical CO_2 extraction of vegetable oils. Journal of Supercritical Fluids 19 (2), 161–175.

Rodrigues, V.M., Sousa, E.M.B.D., Monteiro, A.R., Chiavone-Filho, O., Marques, M.O.M., Meireles, M.A.A., 2002. Determination of the solubility of extracts from vegetable raw material in pressurized CO_2: a pseudo-ternary mixture formed by cellulosic structure + solute + solvent. Journal of Supercritical Fluids, 22 (1), 21–36.

Rosa, P.T.V., Meireles, M.A.M., 2005. Rapid estimation of the manufacturing cost of extracts obtained by supercritical fluid extraction. Journal of Food Engineering 67 (1–2), 235–240.

Sovová, H., Kučera, J., Jež, J., 1994. Rate of the vegetable oil extraction with supercritical CO2–II. Extraction of grape oil. Chemical Engineering Science 49 (3), 415–420.

Sovová, H., Zarevúcka, M., Vacek, M., Stránský, K., 2001. Solubility of two vegetable oils in supercritical CO_2. Journal of Supercritical Fluids 20 (1), 15–28.

Takeuchi, T.M., Leal, P.F., Favareto, R., Cardozo-Filho, L., Corazza, M.L., Rosa, P.T.V., Meireles, M.A.A., 2008. Study of the phase equilibrium formed inside the flash tank used at the separation step of a supercritical fluid extraction unit. Journal of Supercritical Fluids 43 (3), 447–459.

Temelli, F., 2009. Perspectives on supercritical fluid processing of fats and oils. Journal of Supercritical Fluids 47 (3), 583–590.

Yilmaz, Y., Toledo, R.T., 2006. Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols. Journal of Food Composition and Analysis 19 (1), 41–48.